wrench to non-linear dynamical systems such as neuronal and cardiovascular tissues, non-linear optics and chemical reactions, all of which display an excitable binary ('all-or-none') response to input perturbations. Based on this dynamical feature, we devise and implement a conceptually novel sensing technique capable of detecting single perturbation events with high signal-to-noise ratio and continuously adjustable sensitivity.

57-Plat
Single-Molecule Analysis of Intraflagellar Transport in Live Chlamydomonas Cells
Sheng-Min Shih[1], Fatih Kocabas[2], Ahmet Yildiz[1].
[1]University of California Berkeley, Berkeley, CA, USA, [2]University of Texas Southwestern Medical Center, Dallas, TX, USA.
Eukaryotic cilia and flagella are highly specialized for long-distance cargo transportation. Intraflagellar transport (IFT) in *Chlamydomonas* flagella requires two proteins: kinesin II motors that transport submembranous protein particles from the flagellar base to the tip by moving along a unipolar array of microtubules, and IFT dynein motors that recycle these particles back to the cell body. To investigate the interactions between microtubule motors and individual IFT cargos, we have attached fluorescent polystyrene beads coated with an antibody for FMG1, a transmembrane glycoprotein. Multicolor tracking of FMG1-beads and GFP-tagged IFT cargos showed that FMG1 is transported by the IFT machinery. The movement of the FMG1-beads was tracked with 1 nm precision at 400 microsecond temporal resolution. We observed the beads taking 8 nm steps in both the retrograde and anterograde directions. Analysis of bead motion also revealed that IFT cargos move unidirectionally from one end of the flagella to the other with infrequent backward steps. Using an optical trap, we have measured the forces that are exerted on the bead by the motor proteins to estimate the number of engaged motors on single IFT cargos. Beads have stalled near 20-30 pN forces in each direction, indicating that there are at least 4-5 active motors involved in the transportation of IFT cargos. Force measurements in a strain that carries a temperature-sensitive mutant of kinesin II showed that heat inactivation of kinesin II did not alter forces in the retrograde direction. Our results suggest that kinesin and dynein motors do not undergo tug-of-war competition with each other in IFT. We favor a model where the regulation of motors is restricted to the base and the tip of the flagellum to provide a continuous stream of IFT cargos along the flagellum.

PLATFORM C: Muscle: Fiber & Molecular Mechanics & Structure

58-Plat
Ionic Strength Influences the Mechanical Force Regulation of Myosin'S Unbinding Rate
Amy M. Clobes, William H. Guilford.
University of Virginia, Charlottesville, VA, USA.
We previously showed that skeletal muscle actomyosin in various nucleotide states behaves as a catch bond and that bond lifetime is maximal at the isometric force generated by a single myosin molecule. Recent data from our lab suggest the catch-slip behaviour results from allosteric conversion of myosin from a short- to a long-lived bound state. Though these studies shed some light on actin-myosin bond mechanics, the experimental conditions were 5 times lower than physiologic ionic strength. We therefore investigated the possibility that residence times in the short- and long-lived bond state of the myosin head are influenced by ionic strength. Dynamic force spectroscopy was used to measure the load-dependent bond dissociation of nucleotide-free heavy meromyosin (HMM) from actin at 25 mM (typical *in vitro*) and 145 mM KCl (physiologic ionic strength). In this technique, a trapped actin-coated bead was brought into contact with an HMM-coated surface, a bond was allowed to form, and then the bond was loaded until rupture by moving the laser trap away. We also determined bond lifetimes at near-zero load by placing an actin-coated bead adjacent to the HMM-coated surface and allowing bonds to form and break semi-spontaneously. Actin-myosin bond lifetimes were higher on average at physiologic ionic strength compared to low ionic strength across a range of forces. Further, our data suggest that catch bond behaviour is abrogated at physiologic ionic strength.

59-Plat
Actin Sliding Velocities are Influenced by Actin - Myosin Attachment Kinetics
Del R. Jackson Jr., Travis Stewart, Alan Stickney, Josh E. Baker.
University of Nevada, Reno, Reno, NV, USA.
Unloaded muscle shortening velocities (V) are widely thought to be limited by actin-myosin detachment kinetics (T_{on}); however, recent studies indicate that actin-myosin attachment kinetics (k_{att}) significantly influence V. To test the hypothesis that V varies with k_{att}, we use stopped flow fluorescence spectrometry to study effectors of k_{att} and in vitro motility assays to correlate observed effects on k_{att} with changes in V. Here we consider the combined effects of sucrose and potassium chloride, KCl. We show that sucrose specifically inhibits the rate of actin-myosin binding, k_{att}, and the effects of sucrose on k_{att} correlate with its effects on V. Moreover, the addition of KCl enhances the effects of sucrose on k_{att}, with the combined effects of KCl and sucrose on k_{att} mirroring their effects on V, suggesting that the ionic strength-dependence of k_{att} contributes to the ionic strength-dependence of V. We are investigating mechanisms by which both KCl and sucrose influence k_{att}, and we have developed a collective force model that accounts for the observed influence of both k_{att} and T_{on} on V. Our data and model imply that factors that decrease k_{att} such as regulatory proteins, certain disease related mutations, and inhibitors such as blebbistatin can slow V through kinetic rather than recruitment or sequestration mechanisms.

60-Plat
A-Band Shortening in Isolated Myofibrils of Rabbit Psoas Muscles
Maria Yamamoto, Appaji Panchangam, Walter Herzog.
University of Calgary, Calgary, AB, Canada.
Muscle contraction is explained by cyclical interactions between actin and myosin filaments. These filaments are typically assumed to be rigid and thus are not thought to contribute to muscle contraction and force production. However, previous works have observed reduction in A-band width, an indicator of myosin length, in activated single fibers. As individual sarcomeres and A-bands cannot be resolved accurately in single fibers the purpose of this study was to determine A-band widths in a preparation that allows such resolution i.e., isolated myofibrils. Isolated myofibrils (n=13, 10-15 sarcomeres long) of rabbit psoas muscles were placed in a bath containing relaxing solution under an inverted microscope. Myofibrils were secured in two segments with two glass micro needles and a silicon nitrate nano lever. Mean sarcomere length (SL) of the control segment between the glass needle and the cantilever was set to ~2.4μm and the mean SL of the experimental segment between the needles was varied between 2.0μm and 3.2μm. Upon Ca2+-activation, myofibrillar forces were calculated in the control segment from the cantilever deflection (186 ± 112nN). A-band widths and SL were measured from video continually for 5min following activation. Reduction in A-band width (range=0.0-0.6μm) correlated (p=-0.7884) with the post-activation mean SL (range=2.1-3.2μm) of the experimental segment in a bi-linear fashion with an inflection at ~2.8μm. A-bands shorten upon activation at SL below 2.8μm, but remain constant at SL above 2.8μm. This result contradicts long held beliefs about myosin filament rigidity and may challenge our current thinking about the molecular mechanisms of contraction. However, the slow speed of A-band shortening raises questions about its functional relevance in everyday muscle activity and its occurrence at short SL only may suggest a role in adjusting the length of the molecular spring titin.

61-Plat
Sub-Millisecond Time-Resolved 2-Dimensional X-Ray Diffraction Recording from Stretch-Activated Flight Muscle from Bumblebee
Hiroyuki Iwamoto, Naoto Yagi.
SPring-8, JASRI, Sayo-gun, Hyogo, Japan.
We have previously recorded the responses of X-ray reflections from bumblebee flight muscle (IFM) fibers to step length changes at a 3.4 ms time resolution (Iwamoto et al., Biophys. J., 2010). This study showed that, upon stretch, the myosin heads supporting isometric force detached, and were dynamically replaced by new myosin heads recruited by stretch activation (SA). At the same time, tropomyosin molecules (TM) temporarily moved back to its inactivating positions. These motions of proteins were very fast, and clearly the time resolution of the 3-CCD detector (640 x 480 pixels, 3.4 ms/full-frame) was insufficient. Here we used a new high-speed CMOS detector (1024 x 1024 pixels, up to 0.2 ms/full-frame) and repeated time-resolved measurements with a 0.5-ms time resolution. Unlike CCD detectors, the CMOS detector does not have to transmit charges serially, enabling a fast readout. In addition, we have improved the experimental design so that the IFM fibers tolerate higher X-ray dose (thus higher signal). Mitochondrial remnants, a major diffusion barrier for substances, were thoroughly removed to ensure sufficient ATP supply. After these improvements, the analysis from fully Ca-activated fibers showed that the stretch-induced detachment of myosin heads supporting isometric force is complete within 1 ms after completion of a 1-ms stretch, as shown by the reciprocal intensity changes of troponin reflections. The reattachment of myosin heads was slightly faster than the rise of SA force, and the number of attached heads started to decrease while the force was still rising. The movement of tropomyosin was perfectly synchronized with myosin detachment/reattachment, making it likely that tropomyosin and wings are